EVALUATION OF CONDENSED TANNINS IN TEPARY BEAN GENOTYPES

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INTRODUCTION

Limited studies have shown tepary beans (*Phaseolus acutifolius L.*) to have higher levels of zinc and iron (Bhardwaj & Hamama 2004) and lower levels of tannins (Benitez et al. 1994) than common beans (*Phaseolus vulgaris L.*). Since tannins are antinutrients that prevent absorption of iron and iron concentration is a key nutritional trait, this would make tepary bean a good candidate for use in biofortification programs. However little is known about variability for tannin content in tepary bean. Therefore, the objective of this research was to evaluate condensed tannin content in seeds of 26 tepary bean genotypes from the CIAT collection through a butanol-HCl method, developed for common bean and determine diversity for this critical nutritional trait.

MATERIALS AND METHODS

Plant Material: We analyzed 18 cultivated and 7 wild tepary bean genotypes in this study plus a common bean control genotype, ICA Pijao. Samples of 10 seeds from each genotype were used for the analysis. For each of the cultivated accessions, the seed coat was separated by hand and ground for the extraction of tannins; while for each of the wild accessions, whole seed was used, as it was impractical to remove the seed coat from these smaller seeded genotypes.

Extraction procedures: Extraction was carried out with 70% acetone and diethyleter using 10mg of ground seed coat according to Blair et al. (2006). Spectrophotometric detection was via a butanol-HCL method (Porter et al 1986) which extracts most of the tannins present in a sample but which does not guarantee the complete exclusion of other compounds such as anthocyanins or other polyphenols. This occurs because in the reaction with butanol in an acid solution, proanthocyanidins (tannins) are converted to anthocyanidins, which absorb light at 550nm as do Anthocyanins and other related compounds; leading to some overestimation in the quantification. To avoid this, we used a blank to eliminate the matrix effect and at the same time the possible interference from these compounds. The blank is a sample similar to the samples for analysis and treated in the same conditions, but with the difference that in the butanol reaction, this blank is treated with butanol-water and not butanol-HCl.

Calibration curves: The calibration curves used for data transformation from absorbance to percentage of tannins were color curves for *Phaseolus vulgaris* (Blair et al., 2006). Given the predominant colors in the tepary beans that were analyzed the calibration curves for cream and black seeded genotypes were used for cultivated and wild accessions, respectively.

RESULTS AND DISCUSSION

This first attempt to evaluate tannin content in tepary bean was a useful screening of the effectiveness of the butanol-HCl method in this species, along with an initial exploration of the variability of this important nutritional trait. One notable observation was that variability for tepary bean seed coats was similar to previously observed variability in better-studied common beans. The butanol-HCl method was found to be a fast and good alternative to screen samples, however, techniques like HPLC are more sensitive and accurate and would allow a better understanding of qualitative and quantitative profiles of tannins in tepary beans. Our current results can be summarized as follows:

Cultivated tepary beans: We found variability for condensed tannin content among cultivated tepary bean (Table 1) from undetectable levels to 15 mg / g of seed coat for G40022. In comparison, the control common bean genotypes ICA Pijao had total condensed tannins of 16.3 mg / g of seed coat. Color variation for the genotypes used in the analysis ranged from white, yellow, cream, grey to black; with some mixed color or two-tone genotypes. Overall, the highest values for tannin content among the cultivated tepary beans were found in yellow seeded types, which were the predominant type in the survey (white beans were for the most part excluded given that they have very low tannin content which was confirmed by the results presented here). White beans in this study had undetectable levels of condensed tannins. Meanwhile, the cream or brown mottled and black speckled seed types had intermediate values. The range in tannin content found for the 25 genotypes was within the normal range observed for *P. vulgaris* (House et al., 2002), although in general, there was a clear tendency toward low tannin content.

Wild tepary beans: Wild genotypes were complicated to analyze because of their size and in addition all had the same color (grey/black mottled). The total condensed tannin content on a total seed weight basis (rather than on a seed coat basis) was very similar ranging from 0.1 to 4.7 mg/g of seed coat. When assuming approximately 25% seed coat over total seed percentage these values ranged higher than for the cultivated accessions which might be expected due to the darker tones of wild versus cultivated genotypes. Given the similarity between genotypes and the difficulty in estimating an exact conversion percentage it is difficult to make firm conclusions about variation for tannin content in wild tepary beans.

Table 1. Condensed tannin content (mg tannin/g sample) in 25 tepary bean accessions and one common bean control (ICA-Pijao) measured through Butanol-HCl analysis.

Genotype	Status[1]	Color	Soluble Tannins[2]	Insoluble tannins	Total Tannins
G40001	Cultivated	White	0.0	0.0	0.0
G40007	Cultivated	White	0.0	0.0	0.0
G40006	Cultivated	Cream mottled	0.0	1.3	1.3
G40013	Cultivated	Black speckled	5.6	2.9	8.5
G40019	Cultivated	Black	5.1	2.5	7.7
G40021	Cultivated	White	0.0	0.1	0.1
G40022	Cultivated	Yellow	12.2	3.0	15.1
G40025	Cultivated	Yellow	8.1	3.4	11.5
G40033	Cultivated	Yellow	8.5	3.7	12.2
G40037	Cultivated	Yellow	7.0	3.7	10.8
G40066	Cultivated	Yellow	6.5	3.7	10.2
G40068	Cultivated	Yellow	7.4	3.4	10.7
G40084	Cultivated	Brown mottled	2.2	2.5	4.6
G40110	Cultivated	Black speckled	5.2	2.4	7.5
G40112	Cultivated	Yellow	6.3	3.7	10.0
G40161	Cultivated	Yellow	4.6	1.0	5.5
G40200	Cultivated	Brown mottled	7.9	3.0	10.9
G40237	Cultivated	Yellow	8.8	3.5	12.3
G40186	Wild	Grey/Black	0.9	0.6	1.4
G40106	Wild	Grey/Black	nd	0.6	0.6
G40240	Wild	Grey/Black	3.1	1.6	4.7
G40055	Wild	Grey/Black	nd	0.1	0.1
NI576	Wild	Brown/Black	nd	1.1	1.1
P.a 78	Wild	Grey/Black	nd	1.0	1.0
P.a 19	Wild	Grey/Black	nd	1.1	1.1
ICA Pijao	Common bean	Black	14.1	2.3	16.3

1/ Condensed tannins measured in seed coat samples for cultivated tepary and common beans and in whole seed samples for wild tepary beans.
2/ nd = none detected.

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